

BACTERIAL SYSTEMS FOR ANALYZING UBIQUITYLATED POLYPEPTIDES

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 16/322,956, filed on Feb. 3, 2019, which is a National Phase of PCT Patent Application No. PCT/IL2017/050876 having International Filing Date of Aug. 8, 2017, which claims the benefit of priority under 35 USC § 119(e) of U.S. Provisional Patent Application No. 62/371,881 filed on Aug. 8, 2016.

[0002] The contents of the above applications are all incorporated by reference as if fully set forth herein in their entirety.

SEQUENCE LISTING STATEMENT

[0003] The ASCII file, entitled 86367SequenceListing.txt, created on Mar. 22, 2021 comprising 264,259 bytes, submitted concurrently with the filing of this application is incorporated herein by reference. The sequence listing submitted herewith is identical to the sequence listing forming part of the international application.

FIELD AND BACKGROUND OF THE INVENTION

[0004] The present invention, in some embodiments thereof, relates to bacterial systems for analyzing ubiquitylated polypeptides.

[0005] Ubiquitin (Ub) plays a pivotal role in numerous aspects of cellular processes. Therefore, aberrations in the Ub system are involved in a large number of pathologies, including various forms of cancer such as breast and colon cancer, neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, and infectious diseases such as HIV and Ebola. Consequently, there is a critical need for a detailed understanding of the Ub system. Although there have been significant advances in understanding the ubiquitination process, much less is known about the downstream processes. These include substrate recognition by specific enzymatic interactions in the Ub system, and specific interactions between these enzymes and their substrates. In humans, for example, there are 2 E1 Ub-activating enzymes, 34 Ub-conjugating enzymes and more than 600 E3 Ub-ligases. These enzymes work on presumably several thousands of protein substrates, where specificity is mainly achieved by the E2:E3 and E3:Substrates interactions.

[0006] Another factor which impedes the researchers' efforts to fully characterize Ub cascades is the presence of deubiquitinating enzymes (DUBs) which rapidly reverse the ubiquitination signal. The half-life time of ubiquitylated proteins is thus extremely short. Specifically, it has been shown that about 100 DUBs that exist reverse the modification in a highly specific manner.

[0007] Background art includes Keren-Kaplan et al., The EMBO Journal (2012) 31, 378-390 and Su et al., J Immunol 2006; 177; 7559-7566.

SUMMARY OF THE INVENTION

[0008] According to an aspect of some embodiments of the present invention there is provided a method of identifying an agent which regulates the activity or amount of a ubiquitinating enzyme or deubiquitinating enzyme comprising:

[0009] (a) contacting a bacterial cell with the agent, wherein the bacterial cell outputs a detectable or selectable signal which correlates with the ubiquitination level of a substrate; and

[0010] (b) measuring the level or the rate of accumulation of the detectable or selectable signal, wherein a change in the level as compared to the level in the absence of the agent, is indicative of an agent which regulates the activity or amount of the ubiquitinating or deubiquitinating enzyme.

[0011] According to an aspect of some embodiments of the present invention there is provided a method of determining whether an enzyme is capable of ubiquitinating a test substrate, the method comprising

[0012] (a) expressing the enzyme in a bacterial cell;

[0013] (b) expressing ubiquitin in the bacterial cell, wherein the ubiquitin is attached to a first polypeptide fragment;

[0014] (c) expressing the test substrate in the bacterial cell, wherein the substrate is attached to a second polypeptide fragment, wherein the first polypeptide fragment associates with the second polypeptide fragment to generate a reporter polypeptide on ubiquitination of the test substrate; and

[0015] (d) analyzing for the presence of the reporter polypeptide in the bacterial cell, wherein a presence of the reporter polypeptide is indicative that the enzyme is capable of ubiquitinating the test substrate.

[0016] According to an aspect of some embodiments of the present invention there is provided a method of identifying a polypeptide substrate for a ubiquitinating enzyme, the method comprising:

[0017] (a) expressing a plurality of candidate polypeptide substrates in a bacterial cell population, wherein each of the candidate polypeptide substrates is attached to an identical first polypeptide fragment;

[0018] (b) expressing the ubiquitinating enzyme in the bacterial cell population;

[0019] (c) expressing ubiquitin in the bacterial cell population, wherein the ubiquitin is attached to a second polypeptide fragment, wherein the second polypeptide fragment associates with the first polypeptide fragment to generate a reporter polypeptide on ubiquitination of the substrate; and

[0020] (d) analyzing in bacterial colonies of the bacterial cell population for a presence or absence of the reporter polypeptide, wherein a presence of the reporter polypeptide is indicative of expression of a substrate for the ubiquitinating enzyme.

[0021] According to an aspect of some embodiments of the present invention there is provided a kit comprising:

[0022] (i) a first polynucleotide which encodes a first polypeptide fragment which is operably linked to a bacterial regulatory sequence, and a cloning site, wherein a position of the cloning site is selected such that upon insertion of a sequence which encodes a test polypeptide into the cloning site, following expression in a bacterial cell, a fusion protein is generated which comprises the test polypeptide in frame with the first polypeptide fragment; and

[0023] (ii) a second polynucleotide comprising a second nucleic acid sequence encoding a second polypeptide fragment which is attached to ubiquitin, the second nucleic acid sequence being operably linked to a bacterial regulatory sequence, wherein the first polypeptide fragment associates with the second polypeptide fragment to generate a reporter polypeptide dependent on ubiquitination of the test polypeptide.